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ABSTRACT

In this study a Hyperspectral Imaging System (Short Wave Infrared range from 1000 to 2500 nm) was used to model fish texture by experimental compression test. Partial Least Square-Discriminate Analysis modeling technique was used for classifying the samples by linking the hyperspectral information and their measured texture. The R^2 of cross validation and prediction were 0.97 and 0.96, respectively. The Root Mean Squared Errors for cross validation and prediction were 0.07 and 0.09, respectively. Sensitivity and specificity for both class I and II were 1.00. Results indicated that hyperspectral imaging in SWIR range has ability to detect texture stiffness of rainbow trouts which is affected by freshness.

Keywords: Hyperspectral Imaging, Short Wave Infrared, Texture, Compression Test, Rainbow trout

INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*, formerly named *Salmo gairdneri*) is one of the salmonid species which is native to tributaries of the Pacific Ocean in Asia and North America range from Alaska to Mexico (1, 2). The Rainbow trout flesh is tender with a tasty and nutty flavor which made this fish very popular in chain food in all over the world (3). Rainbow trout is one of the major aqua cultural productions in Iran. Annual production of rainbow trout in Iran was 106409 ton, in 2011, which is 13.8% of all production in the world (4).

Texture is a critical parameter that uses to determine the overall quality perception of salmon products (5). Consumers usually accept tenderness rate as the major parameter that determines the quality of meat (6). A primary goal in texture analysis is developing objective measurement methods that accurately define textural characteristics for consumer quality judgments (7). There are several methods for texture analysis of meat and its products. One of them is using a taste panel. But, the design and execution of the taste panels require special skills and experiences for obtaining reliable results (8). On the other hand, the human inspection is a subjective method, which is time-consuming, laborious, tedious, and inconsistent (9). In contrast, texture of salmon fillets can be measured by using mechanical food testing equipment (10). The measurement of texture using an instrument is preferred over sensory evaluations since instruments can reduce variation among measurements and are more precise (11). Some instrumental evaluation methods for meat and its products have been used for texture analysis such as hardness, adhesiveness, cohesiveness, springiness, chewiness, gumminess and fracture ability (12). There are many instrumental methods for measuring the force based on cutting or compressing of a meat sample. However, not all of those techniques are applicable for fish samples, for instance, Shear force is a common method used for pork or beef texture analysis. In Shear force measurement, strips or cores ($1 cm^2 \times 3$ cm) are removed parallel to the muscle fibers and sheared perpendicular to the fibers (12). This technique can't be used for a given fish sample, especially in rainbow trout because it is not possible to remove a core with $1 cm^2 \times 3$ cm dimension. In this situation, the compression test is used instead of Shear force method. By compression test method, sample is compressed to some percentage of its thickness and the maximum force is measured. (12). Beside of this, common traditional method for detection of fish freshness is compression by finger like compression test. If the pressure region returns to the initial state, then this fish is fresh, but if its shape is changed, then this sample isn't fresh. This method is time consuming and depends on individual skills. Because of this, compression test is related to freshness of fish and people use this parameter for detection of sample freshness. However, instrumental methods are destructive, time-consuming and laborious, therefore cannot be used as an automatic system in the industry inspection line.

Hyperspectral imaging (HSI) is a powerful method that has many applications in food products research (13). Hyperspectral imaging has advantages of two different methods namely spectroscopy and imaging (14). In spectroscopy usually one or few points of a given sample is measured while information from the whole sample is measured by a hyperspectral imaging system. This method is nondestructive, fast, and relatively inexpensive. It's applications in agriculture include vegetation mapping, crop disease, stress and yield detection, component identification in plants, and detection of impurities. There is growing interest in HSI for safety and quality assessments of agro-food products (15). Wu et al. applied Partial Least Square Regression (PLSR) to establish a quantitative model between hyperspectral images of salmon fillets and their corresponding parameters of hardness, cohesiveness, and adhesiveness (9). The Correlation Coefficients (r) of 0.665, 0.555 and 0.606 while the Root Mean Square Errors of Cross Validation (RMSECV) of 4.09, 0.067 and 0.504 were achieved for hardness, cohesiveness, and adhesiveness, respectively. The results demonstrated that hyperspectral imaging technique has the potential to quantitatively measure texture of salmon fillet in a rapid and non-invasive way. It was concluded that R^2 for classification is better than regression when the target is the texture analysis of meat product. Liu et al. attempted to classify beef samples into tender and tough classes using spectroscopy which resulted in a model with correct classification of 83% (16). Soft Independent Modeling of Class Analogy of principal component analysis (SIMCA/PCA) model of measured tenderness showed great promise in the classification of tender and tough meats with over 96% success. While the R^2 in regression model was between 0.49 and 0.55 for tenderness and sensory chewiness, respectively. C-1: Not clear?? The Hyperspectral imaging method has acceptable results in other fields such as detection and quantification of ergot bodies in cereals (17), external insect infestations on jujube fruit (18), monitoring quality of tomato fruit (19). The main aim of this research is to model texture of rainbow trout by linking a compression test (as time-consuming and destructive method) and hyperspectral imaging (as fast and nondestructive method) in duration of storage.

MATERIAL AND METHODS

Sample preparation

A total of 80 rainbow trouts were killed, gutted, packed in food plastics individually and then labeled. Samples were divided to four different batches. Each batch had 20 samples and was preserved in ice for 1, 3, 5 and 7 days, respectively. These batches were then stored in 4 boxes full of ice. Fresh ice was added daily to completely cover the fish. The boxes were put in a cold preservation room with 4°C.

Hyperspectral imaging

The hyperspectral imaging was done in HyperSpectral Imaging (HSI) laboratory of MeBioS (Mechatronics, Biostatistics and Sensors) division of Biosystems Department, KU Leuven, Belgium. The hyperspectral imaging system consists of a Short Wave Infrared (SWIR) imager, illumination device and translation stage (Fig. 1). The SWIR hyperspectral imaging was done by using a hyperspectral camera (HS SWIR XS-M320C4-60, Headwall Photonics Inc., Fitchburg, MA) which consists of an MCT camera (XEVA MCT-2140, Belgium) with the optimal sensitivity from 1000-2500 nm and 320 by 256 pixel resolution and a reflective concentric grating (Headwall Photonics Inc., Fitchburg, MA). The spectrograph had a fixed-size internal slit (60 μm) to define the field of view (FOV) for the spatial line. Illumination device consists of a light source with 4 halogen lamps (DECOSTAR ALU 12V 20W 36°, OSRAM, Germany) that were arranged on arc frame to obtain homogeneous illumination of the scanned area. The system operates by push broom camera and scans a single-spatial line of a target object and the spectrograph disperses light from each line element or pixel to a spectrum. Therefore, each image is build based on lines of pixels in one axis and spectral pixels in the other axis. To obtain a three-dimensional (3D) hyperspectral data cube, the sample has to be moved along with the second spatial dimension. A controlled translation stage with adjustable sample holder was used to move the sample using a stepper motor (TLA15-400, Franke GmbH, Aalen, Germany) under the SWIR camera. The system was operated using *Lab View V8.5* software (National Instruments, Austin, TX, USA). The Exposure time was optimized and selected as 9.7 ms in order to have a good signal to noise ratio of the reflectance in whole surface of a fish sample. Translation stage speed and step was 200 mm/s and 0.3 mm, respectively.

Compression test

Compression test was performed by a universal material testing machine equipped with a 200 N load cell (Fig. 2a). The probe was a stainless steel cylinder (diameter: 11 mm). Approximately one-third of the cylinder base was rounded imitating a human finger (Fig. 2b). Sample thickness and the maximum force needed to compress the sample to 40% of its thickness were recorded. The probe compression speed was 1 mm/s. The sample temperature during compression was kept constant at 5–7 ºC by keeping the samples in a plastic film covering with ice until before performing the measurements. An average of three recorded forces performed on each sample was calculated as compression force (12, 20). Statistical analysis was done by applying the analysis of variance (ANOVA) using SPSS13 software. Duncan's multiple ranges test was used to separate means at a 5% level of significance.

Image correction

The dark and white reference images were captured in the optimized data acquisition setup for SWIR camera. A white reference tile (Spectralon® Reflectance Standards, RSS-08-010, Labsphere) with 20% reflectance was used to provide an estimate of the incident light on the tile at each wavelength, which is used in the normalization of the spectra. On the other hand, the dark reference image (~0% reflectance) was obtained by turning off the illumination device together with covering the camera lens completely with its non-reflective opaque black cap. These two images were used for elimination or reducing of the effects of variation in illumination, detector sensitivity and geometry for every pixel in the image using the following equation (21):

$$R(x, \lambda) = \frac{I - I_D}{I_W - I_D} \qquad [1]$$

where, R is the relative reflectance, I is the intensity value captured from the sample, I_{D} is the dark current and I_{W} is the intensity captured from the white reference tile.

Image segmentation and region of interest (ROI) selection

In the captured images, the background, the edges and the saturated pixels were removed before calculating the mean spectrum of each sample. By doing so, these uninformative regions of each fish image which do not carry useful information were removed before data handling. A low and high mask was used in order to remove uninformative regions and select Region of Interest (ROI). Fig.3a shows reflected spectra from the fin and a fish sample using SWIR setup. Reflectance for the background region is zero which is not shown in figure. As shown in Fig.3a, the region between 1054-1150 nm was selected to segment the background, fin edges and fish based on the best differentiation of the mentioned spectra. The threshold value 0.1 was used. Fig.3c shows the selected region in a fish sample (Fig. 3b) by applying low mask with white color. In order to remove the saturated points, a 95% of maximum reflectance (thresholds for high mask) in the 1100 nm bands is used to constrain the high mask. All pixels with value higher than threshold were removed from the images. Fig.3d shows the saturated pixels with white color, detected by applying the high mask criteria. Gray scale area in the Fig.3e shows the interesting region containing of information from fish flesh. Finally applicable region was selected through cutting the head and tail of the fish samples manually (Fig. 3f).

Preprocessing

In order to remove/reduce physical phenomena in the spectra, several preprocessing methods such as smoothing, scatter correction and derivative transformation were used before modeling (22, 23). Savitzky-Golay (SG) function using a window size of 15 was used for smoothing the spectra (24). First and second derivatives are both commonly used in spectroscopy, particularly in near-infrared (NIR) spectroscopy. After smoothing and second derivative function, Standard Normal Variate (SNV) and Multiplicative Scatter Correction (MSC) were used to scatter correction of spectrums (25, 26).

PLS-DA classification

Partial Least Square Discriminate Analysis (PLS-DA) method was used the quantitative regression method of Partial Least Square (PLS) to perform qualitative analysis. The PLS-DA calibration technique links the spectral information with the sample classes. In the model, 60%

samples of each class were used for calibration and 40% samples were used to predict. The Squared Correlation Coefficient (R^2) of the predicted classes versus reference classies obtained by compression test and Root Mean Square Error (RMSE) were used to evaluate the model. R^2 indicates how well predicted and measured class of samples fit a line or curve. RMSE is an estimate of the standard error and was calculated by following equation:

$$\mathrm{RMSE} = \sqrt{1/\mathrm{N}\sum_{\mathrm{i}=1}^{\mathrm{N}}(\hat{\mathrm{y}}_{\mathrm{i}} - \mathrm{y}_{\mathrm{i}})^2} \quad [2]$$

where, $\hat{y}_i$ is the predicted class of the i^{th} sample, y_i is the real class of the i^{th} sample, $\overline{y}$ is the class average of all samples and N is number of samples. Sensitivity and specificity are two statistical measures which are used to investigate the performance of a classification model. The sensitivity of *Class I* is the proportion of *Class I* (Positives) samples that classified as *Class I* (True Positive). The specificity of *Class I* is the proportion of *Class II* (Negatives) samples that classified as *Class II* (True Negatives). These parameters can be written as Eqs. [3] and [4] (27) :

$$\text{Sensitivity} = \frac{\textit{number of True Positives}}{\textit{number of Positives}} = \textit{Probability of Positive test} \quad [3]$$

$$\text{Specificity} = \frac{\textit{number of True Negatives}}{\textit{number of Negatives}} = \textit{Probability of Negative test} \quad [4]$$

Figure 4 shows the flowchart of this research to analysis the data for investigation of storage time effects on rainbow trout texture using hyperspectral imaging. All image processing, modeling and preprocessing methods were performed using PLS-Toolbox (Eigenvector Research Inc, Wenatchee, USA).

RESULTS AND DISCUSSIONS

The mean spectrum of each sample using SWIR hyperspectral imaging system in the ROI by applying low and high masks was calculated. As shown in Fig. 5 the main information of spectra was between 1000 and 1900 nm. For modeling by PLS-DA, the spectra between these regions were used.

Compression test classification

The variance analysis of the obtained maximum force indicated that time storage of rainbow trout has a significant effect on maximum force ($P < 0.01$). Moreover, Table 1 shows obtained results of compression test of rainbow trout for four different storage times of the samples. As indicated, maximum, minimum, mean and standard deviation of 1 day samples is different from other day's samples. In similar research, Liu et al. reported Warner-Bratzler Shear Force (WBSF) for beef meat in duration of ice storage that, the results for 2, 4 and 8 days samples were 7.38±2.28, 5.85±2.06 and 5.36±1.70 kg, respectively (19). As shown, the mean force of 2-days samples is more than 4 and 8 days. Also the difference between 4 and 8 days WBSF is negligible (19). These results confirm our results too.

Fig.6 shows relative frequency of the obtained results by a compression test. As shown, the force measured of samples in the first day of storage is completely separated from those measured in 3, 5 and 7 preservation time. Therefore, 1 day samples assigned as class I and other samples categorized as class II. As described in introduction, one method to detect fresh fish is compressing by finger. If the region which is compressed returns to the initial state, this indicates that freshness of a fish. Now, it can be described that fresh fish has firm texture and needs more force to compress.

PLS-DA modeling

The performance of PLS-DA models was evaluated based on R^2 and RMSE for Calibration (Cal), Cross Validation (CV) and a Prediction (Pre) set. In addition, sensitivity and specificity of models were calculated. The number of latent variable (LV) principle component (PC) was selected 2 based on minimum $RMSE_{CV}$. This model used to detect the class of samples based on compression test classification. Table 2 shows results obtained based on two different preprocessing techniques such as SG+D2+MSC and SG+D2+SNV. As shown in table 2, both techniques resulted in the sensitivity and specificity of 100%. The R^2 of Calibration, Cross Validation and Prediction were 0.97, 0.97 and 0.96, respectively, when SG+D2+MSC was used as preprocessing technique, while 0.98, 0.98 and 0.97 obtained based on SG+D2+SNV,preprocessing technique. This conclusion is according to the previous studies that the performance of models based on both SNV and MSC are rather similar (28, 29). In similar research, classification of tender and tough beef meats reported 0.96 by using WBSF method (19). It should be emphasized that this result obtained using second derivative (D2) that it helps to separate overlapping absorption bands, remove baseline shifts and increase apparent spectral resolution (30, 31).

As a conclusion of paper results, in this research, texture of rainbow trout was analyzed using compression test and hyperspectral imaging. The compression test indicated that texture of fresh samples (or 1 day samples) is firmer than texture of non-fresh samples (or more than 1 day samples) and storage of rainbow trout has significant effect on texture. This study suggests that Short Wave Infrared (1000-2500 nm) hyperspectral imaging system coupled with chemometric techniques has a great potential to be used as a nondestructive, reliable and rapid texture detection tool for rainbow trout. Second Derivative preprocessing combined with as smoothing function and followed by a scatter correction method helps to build a robust model.

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Fig.1. Short Wave Infrared hyperspectral imaging system

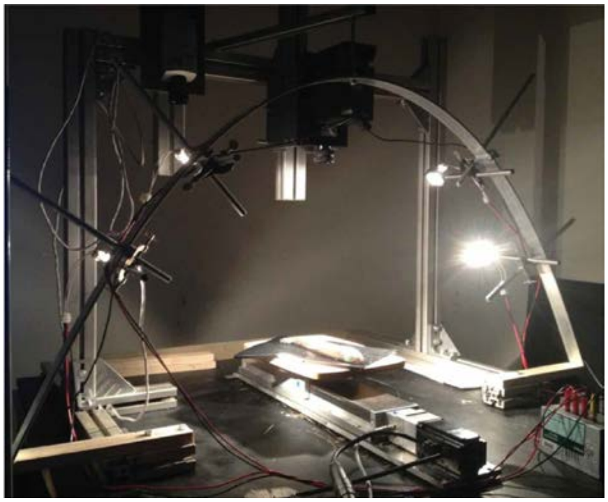

Fig. 2. (a) Compression test using the universal testing machine and (b) stainless steel probe

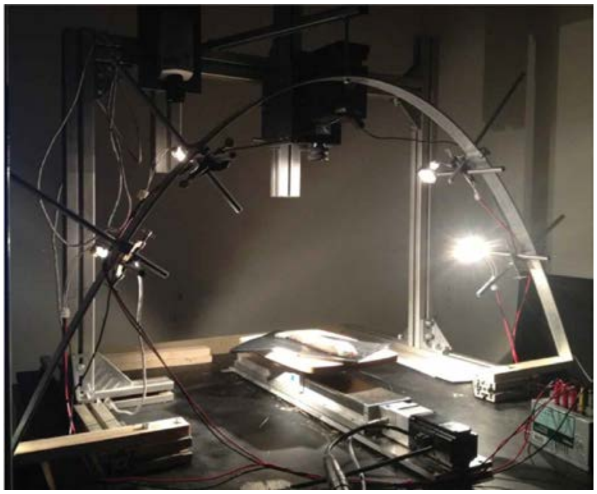

Fig.3. Low and High masks of a fish sample, (a) Reflected spectra from fin and fish, (b) Images in bands 1100 nm, was used to obtain high mask threshold, (c) Image after applying the Low mask, (d) image after applying the High mask, (e) Image after applying the Low and High masks and (f) Final Region of Interest (ROI).

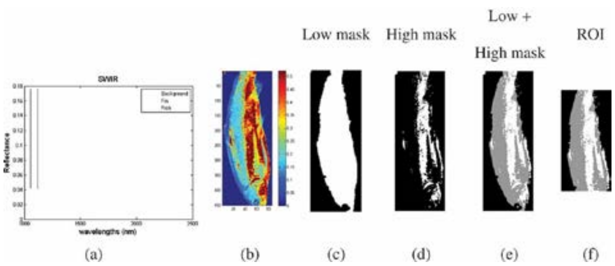

Fig.4. Flow chart of the Texture analysis of rainbow trout using SWIR hyperspectral imaging

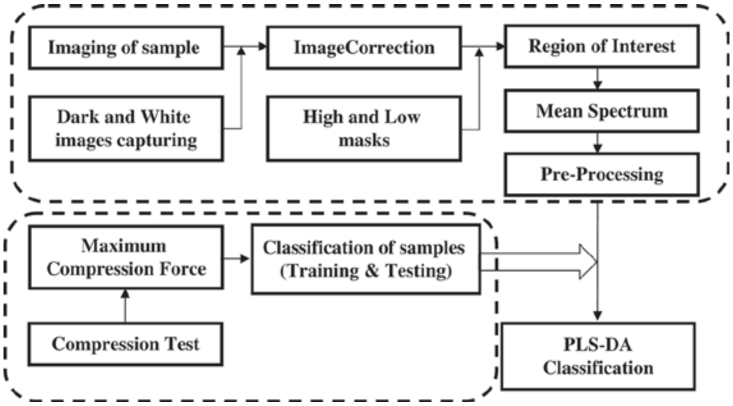

Fig. 5. Raw Spectra of Rainbow Trouts by SWIR hyperspectral imaging

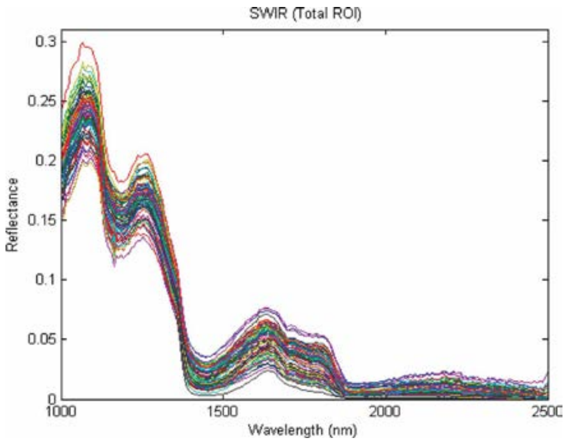

Fig.6. Relative frequency of maximum force obtained from the compression test

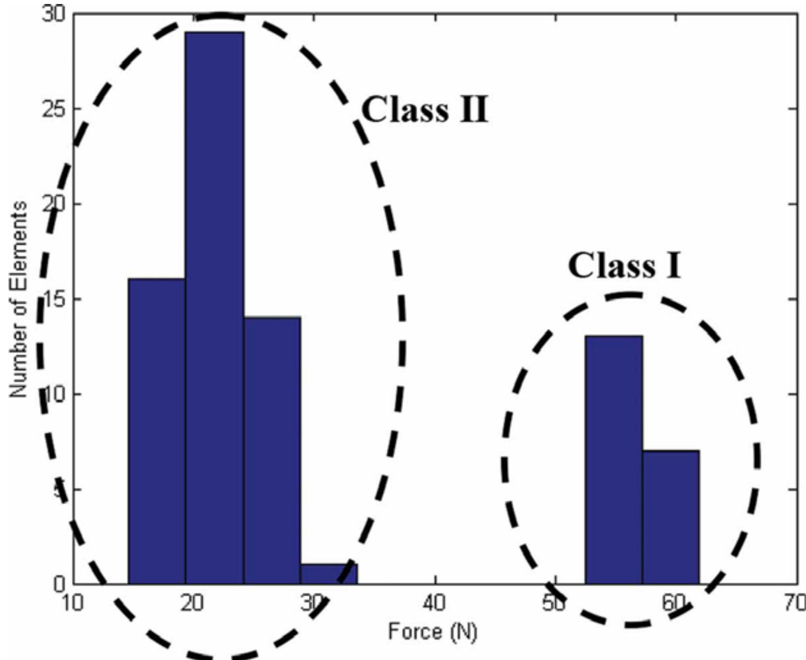

Table 1. Mean comparison of maximum force of rainbow trout for four different storage time batches

Force (N)	1 day	3 day	5 day	7 day
Max	61.98	33.34	27.83	26.12
Min	53.98	16.93	14.66	17.58
Mean	57.15^{a} *	22.63^{b}	20.21^{b}	21.61^{b}
Standard Deviation	1.81	3.93	3.37	2.57

*The means with common letter are not significantly different (P < 0.05)

Table 2. The obtained results by PLS-DA model

Pre-processing	Parameter	Class I			Class II		
		Cal	CV	Pre	Cal	CV	Pre
SG+D2+MSC	Sensitivity	1.00	1.00	1.00	1.00	1.00	1.00
	Specificity	1.00	1.00	1.00	1.00	1.00	1.00
	RMSE	0.06	0.07	0.09	0.07	0.07	0.09
	R^2	0.97	0.97	0.96	0.97	0.97	0.96
SG+D2+SNV	Sensitivity	1.00	1.00	1.00	1.00	1.00	1.00
	Specificity	1.00	1.00	1.00	1.00	1.00	1.00
	RMSE	0.05	0.05	0.07	0.06	0.06	0.08
	R^2	0.98	0.98	0.97	0.98	0.98	0.97